The ET<sub>A</sub> receptor antagonist, BMS-182874, reduces acute hypoxic pulmonary hypertension in pigs in vivo

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Abstract

Objective: Elevated levels of the potent vasoactive peptide endothelin (ET), have been found in pathophysiological conditions associated with pulmonary hypertension. In this study, we have investigated the effects of the ET<sub>A</sub> receptor antagonist, BMS-182874, on hypoxic pulmonary hypertension in pigs. Methods: Pigs were subjected to acute, intermittent 15-min periods of hypoxia FiO<sub>2</sub> 0.1. Following a first hypoxia establishing hypoxic baseline values, vehicle or BMS-182874 10 or 30 mg·kg<sup>-1</sup> i.v. was administered before a second hypoxic period. In separate groups of animals, the effects of the nitric oxide synthase inhibitor N<sub>L</sub>-nitro-L-arginine (L-NNA) in combination with BMS-182874 10 mg during repeated hypoxia were investigated. The ET-1-blocking properties of BMS-182874 were studied in vivo by infusion of ET-1 during normoxia and in vitro using isolated porcine pulmonary arteries. Results: The hypoxia-evoked increase in mean pulmonary artery pressure was reduced by administration of BMS-182874 10 mg·kg<sup>-1</sup> i.v.; from 42 ± 8 to 34 ± 4 mmHg, P < 0.05 and 30 mg·kg<sup>-1</sup> i.v.; from 38 ± 4 to 30 ± 5 mmHg, P < 0.05. In addition, BMS-182874 at 30 mg·kg<sup>-1</sup> reduced the pulmonary vascular resistance during hypoxia from 7.4 ± 1.5 to 5.3 ± 1.1 mmHg·min·l<sup>-1</sup>·P<sub>L</sub> y<sup>-1</sup>; P < 0.05. The hemodynamic response to repeated hypoxia was reproducible in control animals and unaffected by the cyclo-oxygenase inhibitor diclofenac 3 mg·kg<sup>-1</sup>. Infusion of L-NNA alone resulted in an augmented pulmonary vasoconstriction during hypoxia; pulmonary arterial pressure from 35 ± 6 to 43 ± 9 mmHg; P < 0.05 and vascular resistance from 7.2 ± 1.1 to 9.9 ± 1.8 mmHg·min·l<sup>-1</sup>·P<sub>L</sub> y<sup>-1</sup>; P < 0.05. L-NNA in combination with BMS-182874 (10 mg·kg<sup>-1</sup>) resulted in a hypoxic pulmonary vasoconstriction of similar magnitude as hypoxic baseline. In addition, BMS-182874 reduced the hemodynamic response to ET-1 in normoxic pigs and competitively antagonized the vasoconstrictor effect of ET-1 in isolated porcine pulmonary arteries. Conclusions: The non-peptide, selective ET<sub>A</sub> receptor antagonist, BMS-182874, reduces hypoxic pulmonary vasoconstriction in pigs. The reduction in pulmonary vascular response to hypoxia following BMS-182874 is at least partly independent of nitric oxide. © 1998 Elsevier Science B.V.

Keywords: BMS-182874; Endothelin; Hypoxia; Pig; Pulmonary hypertension

1. Introduction

The circulating levels of the potent vasoactive peptide endothelin (ET) [1], have been found to be elevated in pathophysiological conditions associated with pulmonary hypertension [2]. The vascular effects of the isopeptide ET-1 are mediated by at least two classes of endothelin receptors [3,4]; ET<sub>A</sub> receptors, selective for ET-1, causing vasoconstriction and ET<sub>B</sub> receptors, non-selective for the ET isopeptides and proposed to mediate either endothelium-dependent vasodilatation or when located on vascular smooth muscle cells vasoconstriction [5,6]. The most conspicuous effect of ET is sustained vasoconstriction that is slow in onset, in concordance with a regulation of ET release at the level of transcription. Indeed, several studies have indicated that ET-1 may act as a mediator of pulmonary hypertension during chronic hypoxia [7,8], whereas the time course for the contractile effects of ET has been
Arterial and mixed venous blood samples were obtained simultaneously for measurements of blood gas tension and pH. BMS-182874 (generously provided by Dr. Suzanne Moreland, Bristol-Myers Squibb, USA) dissolved in 35 ml 5% NaHCO₃ solution was injected as a bolus dose via the left femoral vein.

ET-1, dissolved in sterile water and angiotensin II (All: Peninsula Labs., UK), dissolved in 0.9% NaCl, were administered through the Swan–Ganz catheter into the right ventricle or tested on isolated pulmonary arteries. L-NNA (Sigma Medical, USA) dissolved in 5% NaHCO₃ solution was infused i.v. via the left femoral vein. Substance P (Peninsula Labs., UK) in 0.9% NaCl was injected as bolus dose i.v. Pigs were left to rest for 30 min after surgery.

2.1. BMS-182874 and controls during normoxia and hypoxia

Following a baseline measurement, the animals were subjected to hypoxia during a 15-min period and the hemodynamic parameters were recorded at the end of this period. Ventilation with room air was thereafter re instituted. After 1 h rest, the animals were randomized to either i.v. bolus injection of vehicle (35 ml 5% NaHCO₃) only (n = 5) or BMS-182874, 10 mg/kg (n = 7) or 30 mg/kg (n = 7). Thirty min after the injections, the protocol was repeated; hemodynamic parameters were obtained during normoxia and at the end of a 15-min period of hypoxia.

2.2. L-NNA during normoxia and hypoxia

In another group of animals (n = 6), hemodynamic recordings were made during basal normoxia and 15 min of hypoxia. After a 75-min resting period, an i.v. infusion of L-NNA (40 mg · kg⁻¹ · h⁻¹) was started. After 15 min of L-NNA infusion, hemodynamic recordings were made first during normoxia, followed by measurements at the end of a 15-min hypoxic period. Substance P, known to induce vasodilatation mainly through endothelial-derived NO production, was given as bolus dose (5 µg) before and during normoxic L-NNA infusion in order to verify the NO-blocking properties of the dose of L-NNA. The rate of the L-NNA infusion was based on preliminary experiments investigating the dose–response curve for L-NNA and substance P in the present animal model (n = 2, data not shown).

2.3. L-NNA and BMS-182874 during normoxia and hypoxia

In a separate group of animals (n = 4), the above protocol using L-NNA (40 mg · kg⁻¹ · h⁻¹) was repeated with the addition of BMS-182874 (10 mg/kg), given i.v. 15 min before L-NNA infusion was repeated. The hemodynamic recordings were then repeated during normoxia and hypoxia.
2.4. Diclophenac during normoxia and hypoxia

In another group consisting of 6 animals, hemodynamic parameters at the end of 15-min periods of hypoxia were recorded before and after administration of diclophenac (3 mg/kg), injected as an i.v. bolus dose 10 min before the second hypoxic period.

2.5. ET-1 infusion after BMS-182874 during normoxia

The dose–response relationship of cumulative infusion of ET-1 (10, 25, 50 and 100 ng·kg\(^{-1}\)·min\(^{-1}\), 10 min each dose) into the right ventricle was established, during normoxia in controls (n = 8) and after an i.v. bolus injection of BMS-182874 10 mg/kg (n = 4).

2.6. All after BMS-182874 during normoxia

To further confirm the selectivity of BMS-182874, the vascular effect of AII was also studied in separate groups of animals. AII was given either as a rapid injection (5 μg) into the right ventricle before and after BMS-182874 (30 mg/kg; n = 8) or as continuous infusion (150 ng·kg\(^{-1}\)·min\(^{-1}\) for 30 min; n = 4) during which BMS-182874 (30 mg/kg) was given after 10 min.

2.7. In vitro experiments

Porcine pulmonary arteries with an inner diameter of 0.8–1.2 mm were obtained immediately postmortem from 6 pigs. The vessels (1–2 mm in length, n = 25) were mounted in 2 ml organ baths using two L-shaped holders [12]. Circular contractions were induced by Tyrode’s solution in which NaCl had been replaced with KCl to give a final concentration of 127 mmol/l. Only vessels responding with two reproducible contractions were used (n = 25). ET-1 (10\(^{-10}\) to 10\(^{-7}\) mol/l) was added to the organ baths in a cumulative fashion in controls as well as vessels incubated for 20 min with BMS-182874 10\(^{-6}\) or 10\(^{-5}\) mol/l.

2.8. Statistics

Results are presented as means ± standard deviation. Ordinary or repeated analysis of variance (ANOVA) followed by a Bonferroni multiple comparison test or the Student’s t-test for unpaired samples were used for statistical evaluation, (GraphPad Software, Instat 2.01). P < 0.05 was considered significant.

3. Results

3.1. BMS-182874 and controls during normoxia and hypoxia

In control animals (Table 1a), hypoxia (arterial pO\(_2\), 24 ± 8 mmHg) induced a reproducible increase in MPAP and PVR, which was reversed during normoxia. In animals receiving BMS-182874 at either 10 or 30 mg/kg (Table 1b,c), the first period of hypoxia (i.e. no BMS-182874 present) evoked a similar increase in MPAP and PVR.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normoxia 1</th>
<th>Hypoxia 1</th>
<th>Normoxia 2</th>
<th>Hypoxia 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>115 ± 13</td>
<td>97 ± 11</td>
<td>121 ± 23</td>
<td>105 ± 17</td>
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<tr>
<td>MPAP (mmHg)</td>
<td>18 ± 3</td>
<td>38 ± 4**</td>
<td>20 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>8 ± 1</td>
<td>9 ± 2</td>
<td>9 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>8 ± 2</td>
<td>9 ± 3</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>3.4 ± 0.4</td>
<td>4.1 ± 1.0</td>
<td>3.6 ± 0.6</td>
<td>4.1 ± 1.1</td>
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<td>SVR (mmHg·min(^{-1}))</td>
<td>32 ± 4</td>
<td>22 ± 4*</td>
<td>32 ± 6</td>
<td>24 ± 8</td>
</tr>
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<td>PVR (mmHg·min·L(^{-1}))</td>
<td>2.9 ± 0.5</td>
<td>7.4 ± 2.1*</td>
<td>3.0 ± 0.4</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>86 ± 16</td>
<td>110 ± 25</td>
<td>98 ± 28</td>
<td>111 ± 26</td>
</tr>
</tbody>
</table>

* Data are presented as means ± s.d.
** P < 0.05, *** P < 0.01, **** P < 0.001. Ordinary measures of analysis of variance (ANOVA): (a) hypoxia 1 and normoxia 2 compared to baseline normoxia (normoxia 1), hypoxia 2 compared to hypoxic baseline (hypoxia 1); (b) and (c) animals receiving BMS-182874, normoxia 2 compared to normoxia 1 and values during hypoxia 2 compared to first hypoxic period (hypoxia 1).
which returned to baseline values during normoxia. In the pigs receiving BMS-182874 at 10 mg/kg, the second period of hypoxia resulted in a significantly lower MPAP compared to hypoxic baseline (Table 1b). In addition, the higher dose of BMS-182874 (30 mg/kg; Table 1c) resulted in a decrease of both the MPAP and PVR during the second hypoxic period compared to hypoxic baseline. In the systemic circulation, hypoxia resulted in a reduction in SVR, which was not significantly altered after administration of BMS-182874, while the MAP during hypoxia was reduced after BMS-182874 at 30 mg/kg. BMS-182874 (10 or 30 mg/kg) did not induce any significant hemodynamic alterations during normoxia, although a tendency to lower PVR was noted. BMS-182874 administration did not change arterial or venous blood gases (pH, PO₂, pCO₂, not shown) during normoxia or hypoxia. Vehicle was found to have no effects on hemodynamic parameters or blood gases.

3.2. L-NNa during normoxia and hypoxia

Compared to basal conditions, infusion of L-NNa (40 mg·kg⁻¹·h⁻¹) did not evoke any significant hemodynamic changes during normoxia (Table 2a). However, L-NNa infusion during the second hypoxia resulted in an increase in MPAP and PVR compared to first hypoxic period. In the systemic circulation, L-NNa infusion elevated MAP during hypoxia. Substance P at a dose of 5 μg evoked a prompt reduction of the MAP and SVR by 4% reduction of MAP and 9%, respectively. After administration of L-NNa the effect of substance P was highly reduced (4 ± 4% reduction of MAP P < 0.001 and 3 ± 6% increase in SVR; P < 0.01 compared to control).

3.3. L-NNa and BMS-182874 during normoxia and hypoxia

Infusion of L-NNa in the presence of BMS-182874 (10 mg/kg) did not evoke any hemodynamic changes during normoxia (Table 2b). However, during the second hypoxia

<table>
<thead>
<tr>
<th>(a)</th>
<th>Normoxia 1</th>
<th>Hypoxia 1</th>
<th>Normoxia 2</th>
<th>Hypoxia 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>111 ± 22</td>
<td>92 ± 8</td>
<td>116 ± 10</td>
<td>116 ± 9 *</td>
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<tr>
<td>MPAP (mmHg)</td>
<td>23 ± 3</td>
<td>35 ± 6</td>
<td>23 ± 3</td>
<td>43 ± 9 *</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>11 ± 2</td>
<td>11 ± 1</td>
<td>10 ± 2</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>8 ± 1</td>
<td>10 ± 1</td>
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<td>10 ± 1</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>3.1 ± 0.6</td>
<td>3.3 ± 0.8</td>
<td>3.2 ± 0.9</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>SVR (mmHg·min·L⁻¹)</td>
<td>34 ± 7</td>
<td>25 ± 5</td>
<td>36 ± 6</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>PVR (mmHg·min·L⁻¹)</td>
<td>3.9 ± 1.0</td>
<td>7.2 ± 1.1</td>
<td>4.4 ± 0.9</td>
<td>9.9 ± 1.8 *</td>
</tr>
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<td>HR (beats/min)</td>
<td>96 ± 10</td>
<td>101 ± 23</td>
<td>89 ± 9</td>
<td>100 ± 24</td>
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<tr>
<td>(b)</td>
<td>L-NNa + BMS-182874</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>107 ± 8</td>
<td>98 ± 10</td>
<td>119 ± 21</td>
<td>92 ± 10</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>21 ± 2</td>
<td>38 ± 4</td>
<td>19 ± 1</td>
<td>38 ± 7</td>
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<td>PCWP (mmHg)</td>
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<td>CVP (mmHg)</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.5</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>SVR (mmHg·min·L⁻¹)</td>
<td>29 ± 4</td>
<td>27 ± 4</td>
<td>34 ± 5</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>PVR (mmHg·min·L⁻¹)</td>
<td>3.6 ± 0.6</td>
<td>8.6 ± 1.1</td>
<td>3.2 ± 0.5</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>93 ± 10</td>
<td>100 ± 10</td>
<td>82 ± 5</td>
<td>108 ± 20</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviation. * P < 0.05. Ordinary measures of analysis of variance (ANOVA), normoxia 2 compared to normoxia 1 and values during hypoxia 2 compared to hypoxic baseline (hypoxia 1).
(i.e. BMS-182874 and l-NNA present), the augmented hypoxic pulmonary vasoconstrictive, evident in the group of animals receiving l-NNA infusion alone (Table 2a), was reduced (Table 2b; hypoxia 2).

3.4. Diclophenac during normoxia and hypoxia

Intravenous administration of diclophenac (3 mg/kg) did not affect the hemodynamic parameters during normoxia or hypoxia. The pulmonary hypertensive effect of hypoxia remained unchanged (MPAP 37 ± 6 mmHg; PVR 6.9 ± 1.2 mmHg·min⁻¹ before and MPAP 38 ± 6 mmHg; PVR 7.4 ± 2 mmHg·min⁻¹ after diclophenac).

3.5. ET-1 infusion after BMS-182874 during normoxia

Cumulative infusion of ET-1 resulted in a dose-dependent increase in PVR and SVR, while the CO decreased (Fig. 1). At the highest dose (100 ng·kg⁻¹·min⁻¹) the ET-1 induced increase in PVR and SVR was attenuated in the animals receiving BMS-182874 compared to controls.

3.6. AII after BMS-182874 during normoxia

Bolus injection of AII (5 µg) evoked an increase in MAP (from 120 ± 14 to 151 ± 14 mmHg) and MPAP (from 19 ± 4 to 26 ± 4 mmHg), which remained unchanged after BMS-182874 administration (MAP 147 ± 11 mmHg and MPAP 24 ± 3 mmHg, respectively). Continuous infusion of AII (150 ng·kg⁻¹·min⁻¹ for 30 min) resulted in a stable, sustained increase in the pulmonary vasotonus (after 10 min of infusion; MPAP 169 ± 11% and PVR 153 ± 23% of values before infusion), which was not altered by BMS-182874 (20 min after BMS-182874 at 30 mg/kg; MPAP 155 ± 12% and PVR 148 ± 19% of control values before AII infusion).

3.7. In vitro experiments

Potassium (127 mmol/l) evoked a strong contraction (5.8 ± 1.6 mN) of the isolated porcine pulmonary arteries which was unaffected by incubation with BMS-182874 (101 ± 6 and 109 ± 13%, at BMS 10⁻⁶ or 10⁻³ mol/l compared to K⁺-induced contractions, respectively). ET-1 caused a concentration-dependent contraction of the pulmonary arteries, which was dose-dependently attenuated by incubation with BMS-182874 (Fig. 2). The maximum response at the highest concentration of ET-1 was not altered by BMS-182874.

4. Discussion

Our present data show that ET₄ receptor antagonism with BMS-182874 can cause a dose-dependent, significant reduction of MPAP and PVR during hypoxia. The ET receptor blocking properties of BMS-182874 was apparent both in vivo and in vitro, while no effect was observed on the response to AII or K⁺. The in vitro experiments showing unaffected maximum response of ET-1 after BMS-182874 suggests competitive binding to the ET₄ receptor [11]. Although BMS-182874 has previously been shown to influence thromboxane A₂ binding [11], the use of diclophenac in a concentration known to block cyclooxygenase activity [13] suggests that an involvement of cyclo-oxygenase products formed during hypoxia in the presently used experimental model is unlikely.

Our findings support earlier studies showing that the selective ET₄ receptor antagonist, BQ-123, reduces the pulmonary vasoconstriction during acute hypoxia in mature fetal lambs [14] and in the rat [15], which has also been shown for pulmonary hypertension evoked by chronic hypoxia [16]. Other studies have shown no effect of BQ-123 on hypoxia-induced vasoconstriction in isolated rat lung [17] or in the intact newborn lamb [18]. These contradictory findings could be explained by differences between the species studied, age of the animals and experimental model employed.

It can be argued that although BMS-182874 evoked no clear-cut changes in vascular tonus during normoxia, there was a tendency to pulmonary vasodilatation and these changes in baseline tonus would alter the vascular response to hypoxia. The experimental protocol was designed to exclude variations in baseline values and responses to hypoxia between different groups of animals. Consequently, all conclusions were made from changes within each group of animals. The hypoxic measurements were made at the end of each hypoxic period and are not likely to be explained by values during the normoxia preceding 15 min of hypoxia. However, a limited influence of the lower normoxic baseline on the magnitude of hypoxic response cannot be excluded.
L-NNA elevated both pulmonary and systemic vascular tone during hypoxia, in concord with previous studies using nitric oxide synthase inhibitors in the pig [19]. Although hypoxia has been shown to reduce endothelium-derived NO activity in the rat [20], our data demonstrating an increase in hypoxic pulmonary vasoconstriction after inhibition of NO support that endogenous NO acts as a pulmonary vasodilator during acute hypoxia [19].

Earlier studies have shown that ET, by activation of ET$_B$ receptors, can induce vasodilation through the release of NO and prostacyclin [21,22]. Our data using diclofenac indicate that vasodilatation, through the release of prostacyclin during hypoxia, is of less importance in the model studied. Furthermore, BMS-182874 in combination with L-NNA resulted in a hypoxic pulmonary vasoconstriction of similar magnitude as hypoxic baseline, whereas the group of animals receiving L-NNA alone demonstrated an augmented hypoxic response during the second hypoxic period. These data indicate that the effects of BMS-182874 are at least partly independent of NO production. However, the complex interactions between ET and NO pathways may also contribute to these results. Endothelial-derived NO has been described to reduce ET-1 production in the porcine aorta in vitro [23]. NO inhibition could thereby result in an up-regulation of the ET pathway during hypoxia. In addition, it is possible that ET$_A$ antagonism could result in an up-regulation of the NO pathway. We have, in a recent study found, that the vasodilator effect of exogenously administered ET-1 during hypoxia is attenuated by the selective ET$_B$ receptor antagonist, BQ-788, although the hypoxic response per se was not affected by ET$_B$ antagonism [24]. A reduction of the hypoxic pulmonary vasoconstriction following ET$_A$ receptor blockade could theoretically be attributed to an inhibition of ET$_A$ receptor-mediated vasoconstriction, thereby unmasking the vasodilator effect of ET$_B$ receptors on the vascular endothelium. However, our present data do not indicate that the observed effects of ET$_A$ receptor antagonism on hypoxic pulmonary hypertension are dependent on an indirect effect on ET$_B$ receptors, since infusion of L-NNA did not abolish the vasodilator effect of BMS-182874 and diclofenac was found to have no effect during hypoxia. Moreover, we have, in a previous study, found that the non-selective ET receptor antagonist, bosentan, also reduces the hypoxic pulmonary vasoconstriction in the pig [25].

In humans, the circulating plasma levels of ET-1 are low during normal conditions, but elevated in a variety of pathological conditions including pulmonary hypertension (see [26]). Interestingly, mountaineers exposed to hypoxia at high altitude developed, within 22 h, elevated plasma ET-1 levels that correlated with estimated MAP, indicating that ET may play a role in acute hypoxia in humans [27].

In conclusion, the present study shows that the non-peptidergic ET$_A$ antagonist, BMS-182874, can reduce the pulmonary hypertension in a large animal in vivo model of acute hypoxia, supporting the idea that ET may participate in pathological conditions associated with elevated PVR due to hypoxia.

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References


